

Mehrdad Arshadi 2018-06-12, Umeå

Separation of Biocides from fermented rye bran

Procedure: In order to separate some compounds in the solution SPE used according to the procedure below:

Solid phase extraction (SPE)

The supernatant of the solution with biocides (from Torgny) was fractionated on a solid phase extraction column: Strata C18-E (55µm, 70 Å) 10g/60 mL, Giga Tubes (Phenomenex). The column was activated with 20 mL of methanol and equilibrated with 20 mL of 10 mM sodium acetate (pH 4.0)¹. 25 mL of sample (supernatant) was load on the column. Thereafter washed with 20 mL of 5% (v/v) aqueous acetonitrile and then eluted by 20 mL of 30% flowed by 20 mL of 60% and 20 mL of 90% aqueous acetonitrile. It will be four fractions totally.

The four eluted and 20 mL of supernatant were concentrated by vacuum (26h at 25°C) evaporation in a Buchi Syncore apparatus. All five concentrated samples were analyzed for antimicrobial activity. We recommend to use Luna Omega 3 um polar C18 100A(LC column 100 x 4.6 mm) from Phenomenex. The column is available for the project.

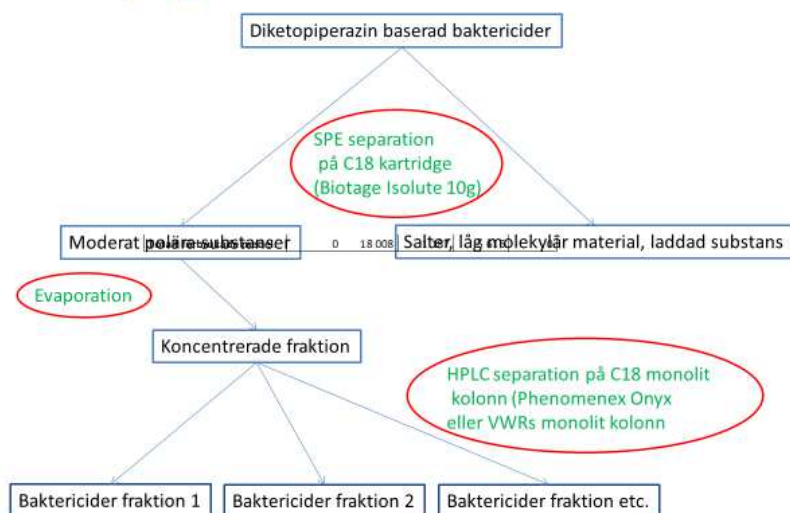
The procedure is a modified version of the attached document:

E.J. Yang, H.C. Chang, Purification of a new antifungal compound produced by *Lactobacillus plantarum* AF1 isolated from kimchi, International Journal of Food Microbiology 139(1) (2010) 56-63.

Notes:

1 It is important to have correct pH of sodium acetate (please check the pH before use it). It was prepared by: $\text{pH} = \text{pKa} + \log(\text{acetate concentration}/\text{acetic acid concentration})$; $\text{pKa} = 4.77$, acetate concentration/acetic acid concentration = 0.169.

Proof of principle



Figuren visar en schematisk illustration av SPE-separation som utförts på fermenterad rågkli.